Demo: Creating the Calibration Curve and Processing Method 8261 Data (PC version)

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Demonstration. Install SMCREPORTER

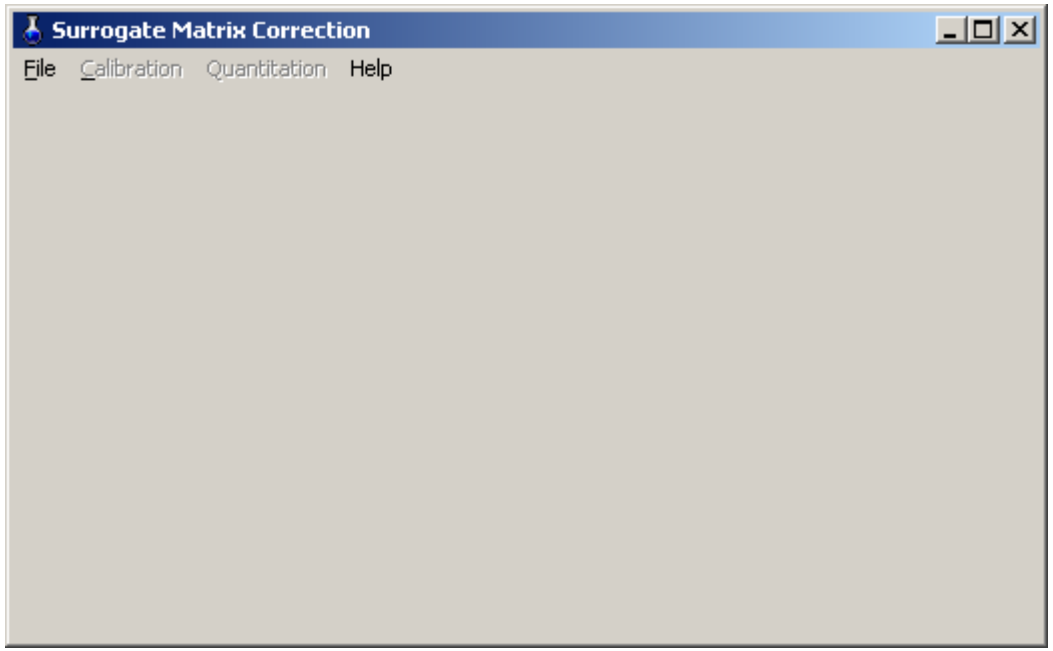
- This slide show is in addition to documentation provided with method 8261 software.
- The software is available on this website http://www.epa.gov/nerlesd1/chemistry/vacuum/methods/software-pc.htm
- Download the zip file as per Installation Guide at the site (It is recommended to also download the Getting Started and User's Guide but not necessary for this exercise).
- You will have created a folder "SMCREPORTER" on the C drive as the default.

Load Demonstration Files for this Exercise

- The reader can reproduce the processing to be presented by installing Smcreporter-Standalone and installing the data files used. Both can be installed from http://www.epa.gov/nerlesd1/chemistry/vacuum/methods/default.htm.
- If the software and data files are installed a new folder, SMCREPORTER, is created. Under the new folder a sub-folder Cal_example contains the example data files including the method 8261 library (All.txt), surrogate file (surrogate.ini), blank (blank.txt), and five standards (StandardA through StandardE).
- **This presentation assumes an understanding of the method 8261 calculations using surrogates**. See http://www.epa.gov/nerlesd1/chemistry/vacuum/reference/analysis/anal.htm .

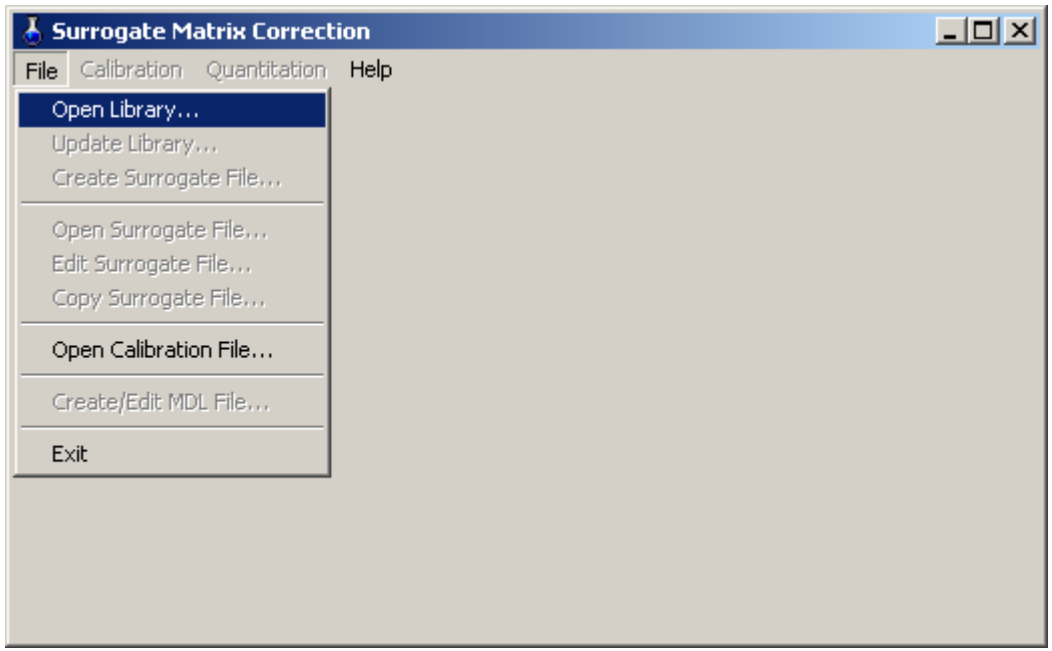
Run SmcReporter

- Go to Windows->Programs->and click on SmcReporter. The following is displayed



Load Library "ALL.txt"

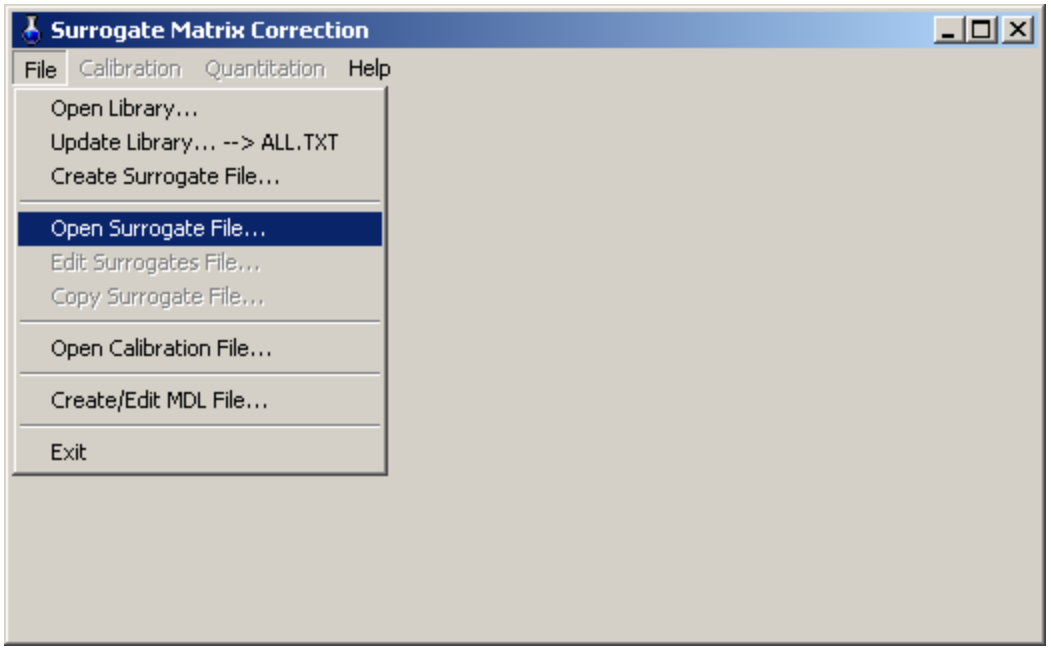
- Go to File then "Open Library"



- Open File window is displayed. Navigate to the Cal_example folder and highlight All.txt and Open. The "All" library is now activated.

Load Surrogate File "surrogate.ini"

- Go to File then "Open Surrogate File"



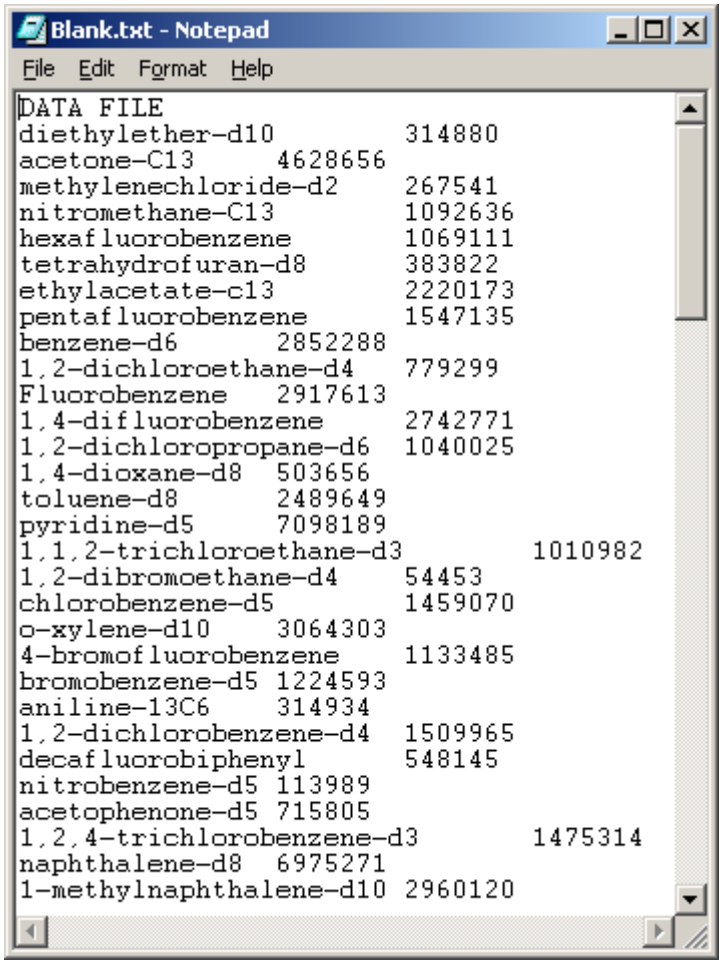
- Open File window is displayed. Navigate to the Cal_example folder and highlight surrogate.ini and Open. The surrogate file is activated.

GC/MS data set

- The PC version data must be in ASCII format and readable in Notepad (e.g. StandardA.txt)
- These data files contain the GC/MS response (area) for each compound.
- Each compound in the “All.txt” library must be present in the data file.
- The first line of the data file contains the label “DATA FILE”

Data File Example

- Open the blank.txt data file in notepad
- Note each compound is identified with a GC/MS response tab delimited

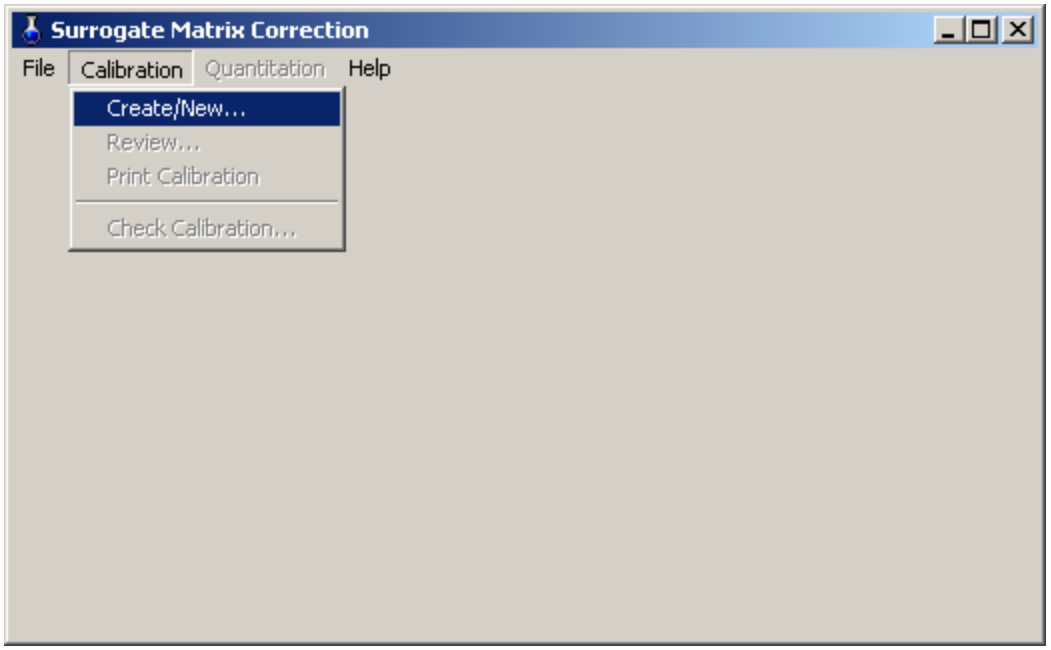


Calibration

- The library and surrogate files represent the compounds in the surrogates and standards (see slide presentation “Running Samples”)
- Standards are prepared as in “Running Samples” slide presentation (http://www.epa.gov/nerlesd1/chemistry/vacuum/training/pdf/run_samples.pdf) with the exception the nitrogen containing compounds are 3X the amounts used in “Running Samples”.
- The standard amounts for Standards A through E are expressed as dilutions of conc1 in the All.txt library (the mass is by compound and not global)
- All runs contain 5 uL of the working surrogate solution and standards are as follows:
    - A. 5uL 1:1 working standard, Conc1 or dilution 1
    - B. 1uL 1:1 working standard, dilution 0.2
    - C. 5uL 1:10 working standard, dilution 0.1
    - D. 3uL 1:10 working standard, dilution 0.06
    - E. 1uL 1:10 working standard, dilution 0.02

Creating the Calibration Curve for Data Processing

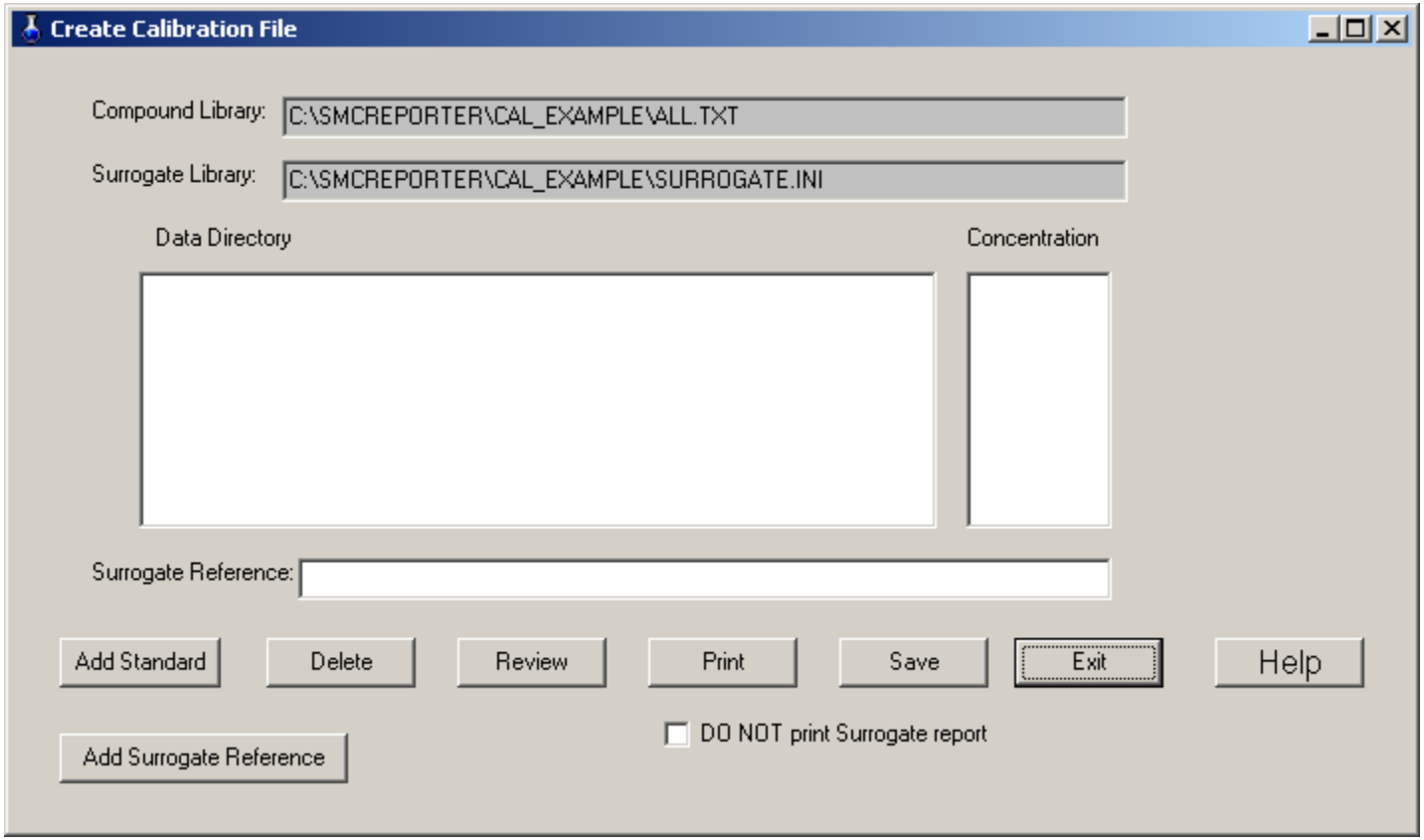
- Go to Calibration and select “Create/New”



- A display indicates library and surrogate files that are going to be used for calibration. Verify “surrogate.ini” and select “OK”

Create Calibration File

- The next window, “Create Calibration File” is then displayed.

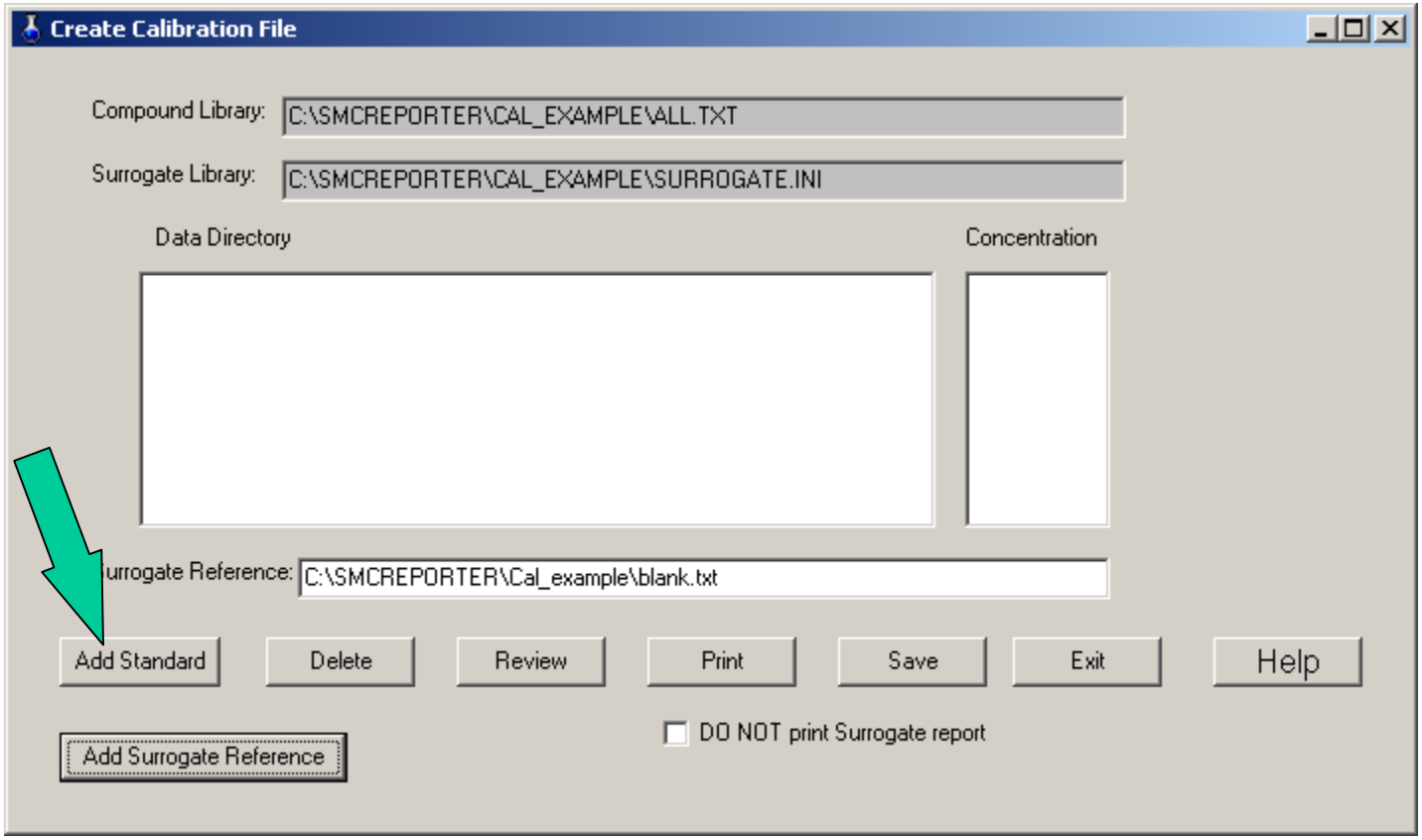


Surrogate Reference

- The surrogate reference used in calibration is a list of surrogates with their boiling points (BP's) and relative volatilities (α) and how these surrogates are to be used.
- Surrogates monitor variations and their responses are used to normalize standard's response factors.
- A surrogate reference file can be any of the runs used to generate a calibration.
- Typically the blank run (blank.txt) run prior to the calibration standards is used.
- Select "Add Surrogate Reference" and using the "Open" window that is displayed navigate to the Cal_example folder and select blank.txt file.

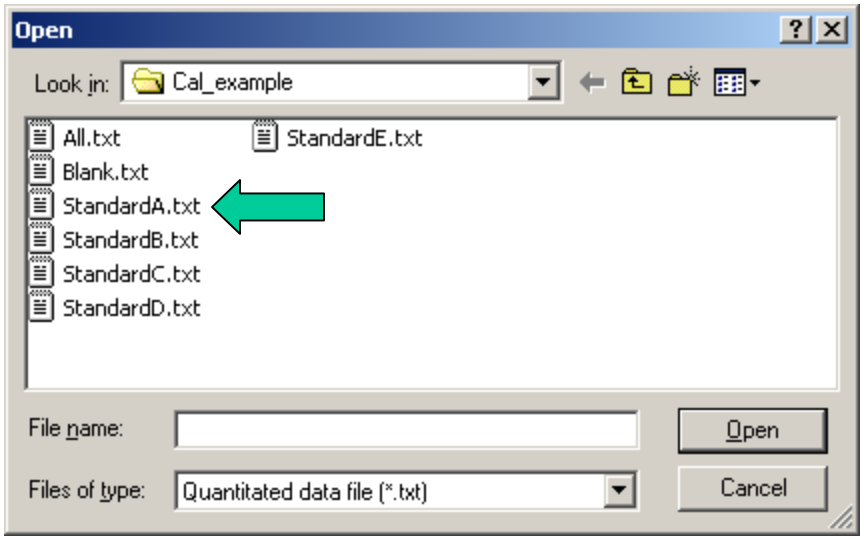
Create Calibration File with Surrogate Reference Added

- Click on “Add Standard.”



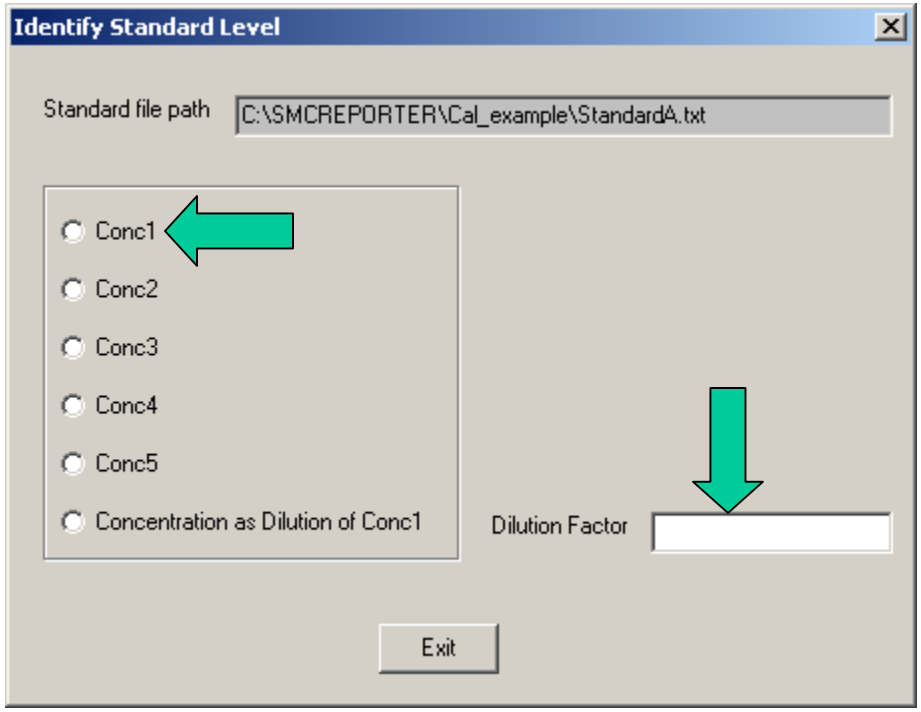
Add Standard "A"

- Using the "Open" window navigate to the Cal_example folder and select the file StandardA.txt then open. This file is the standard "A" listed in slide #9. Click "Open."



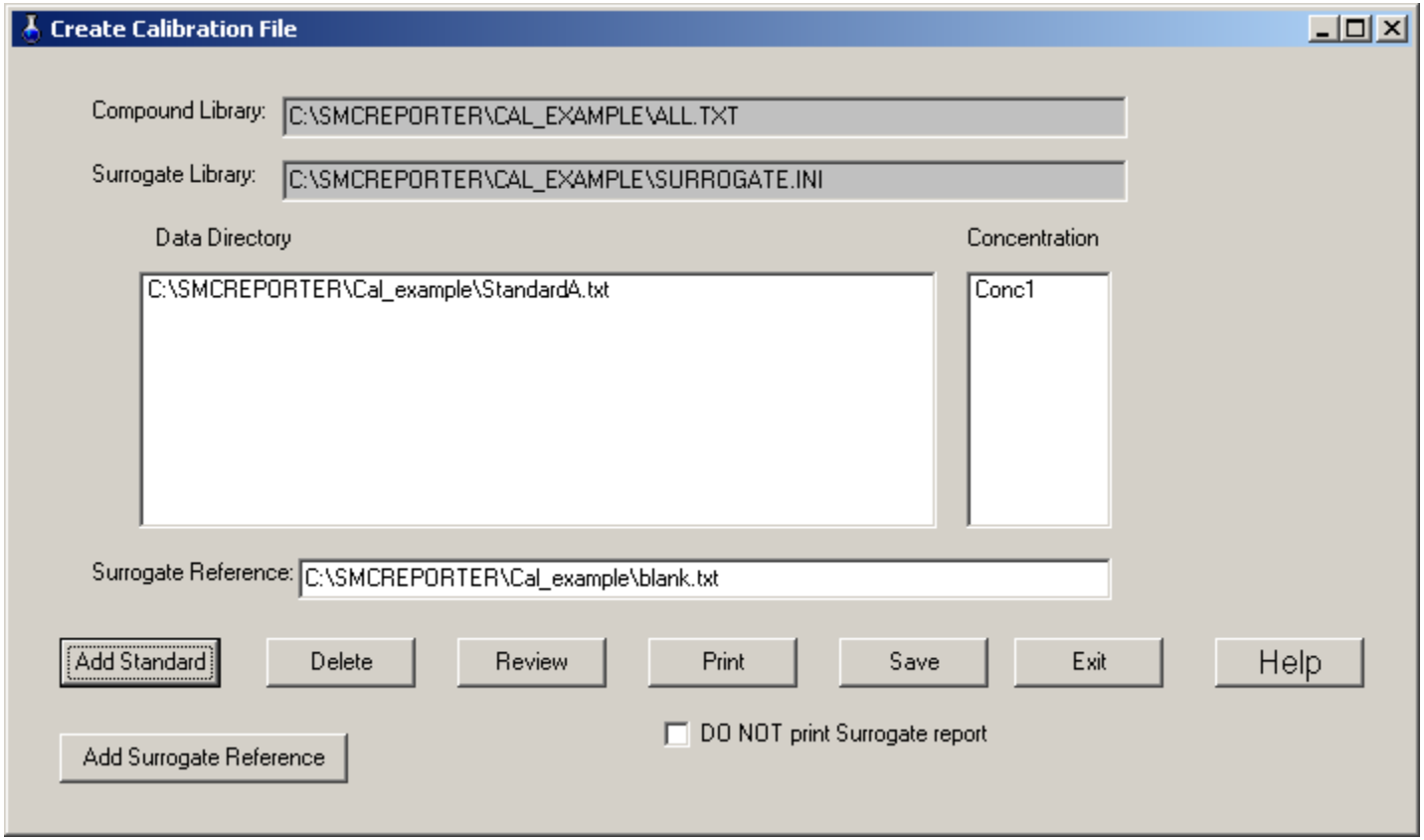
Adding Standards

- The Identify Standard Level window is displayed
- Select Conc1 or enter 1 in the Dilution factor field
- If a dilution factor is entered the Concentration as Dilution of Conc1 is automatically toggled.
- Select Exit to enter



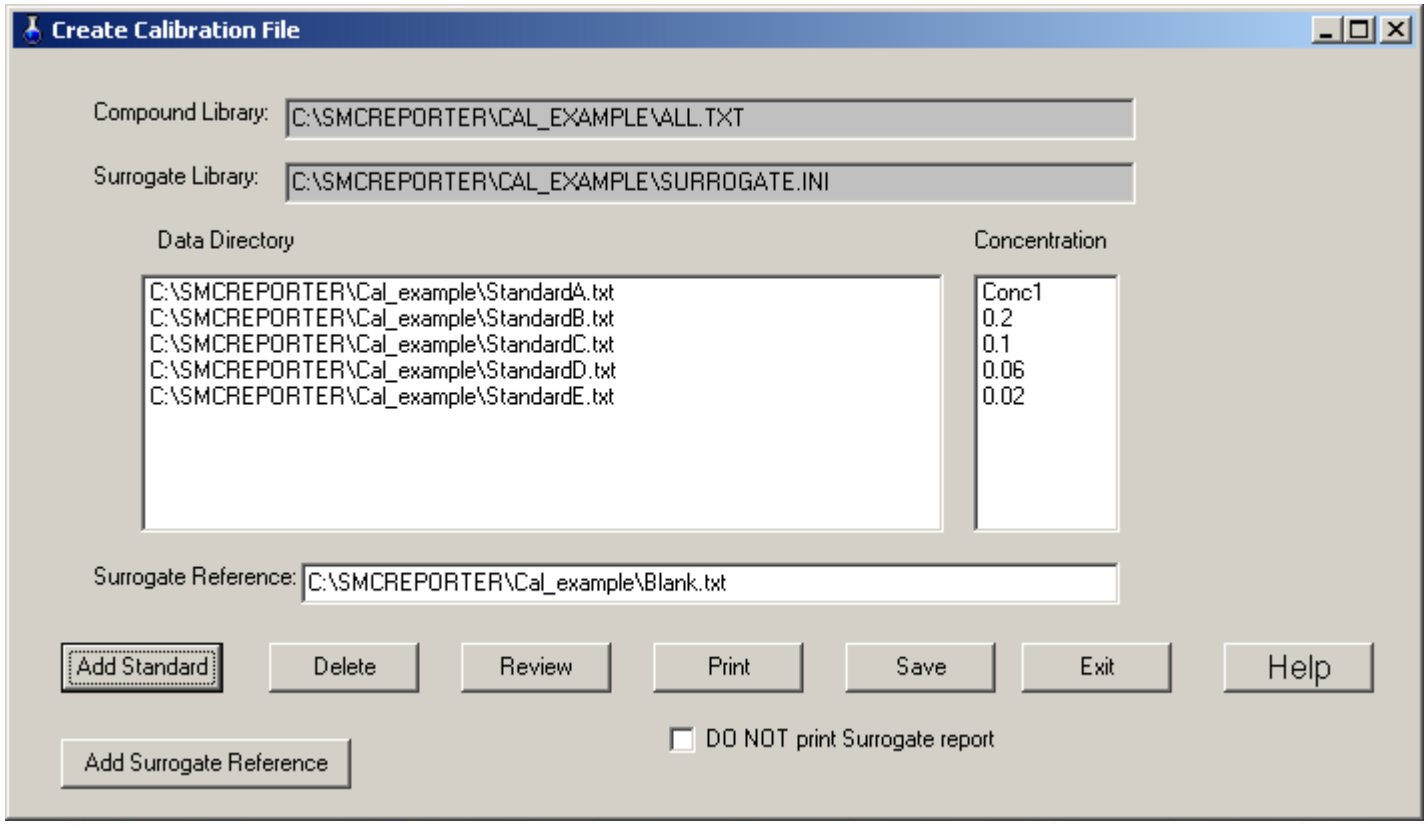
Create Calibration File with Standard A Added

- If there was an error in entry, highlight the standard file in the Data Directory and press “Delete” and repeat the Add Standard routine



Add Standards B-E

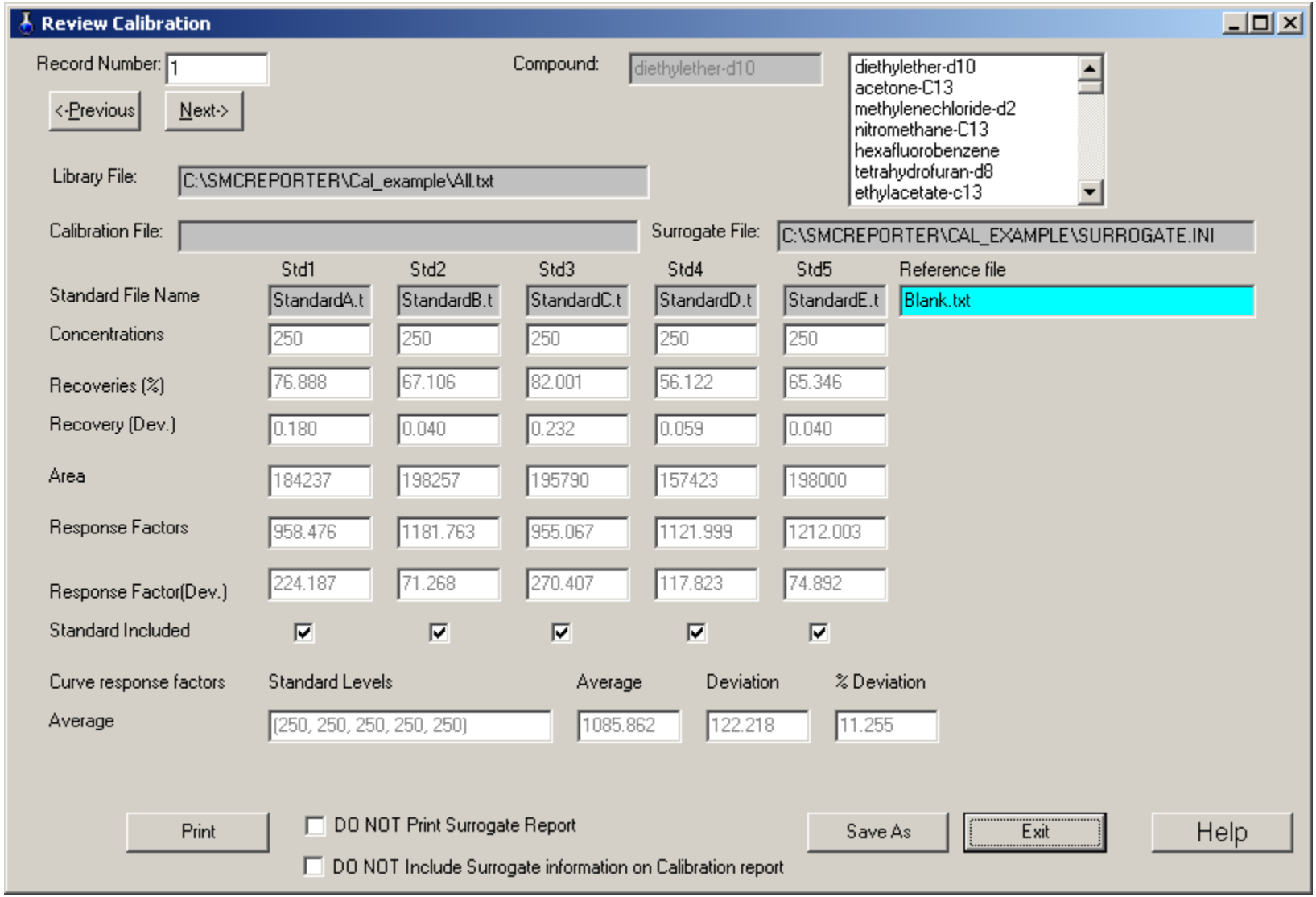
- Repeat the “Add Standard” routine for standards B-E. The dilutions for these standards are (0.2, 0.1, 0.06, and 0.02 respectively.
- The Create Calibration File should appear as



Review calibration curve

- If all entries are correct the next step is to review the data.
- Press the “Review” tab on the “Create Calibration File” form.
- The following slide presents the “Review Calibration” form, starting at the first library entry (the default).
- Note that no sample sizes are entered. Only the total ngs are being displayed. This is an important feature of method 8261 feature in that calibrations are by mass and not by matrix or sample size.

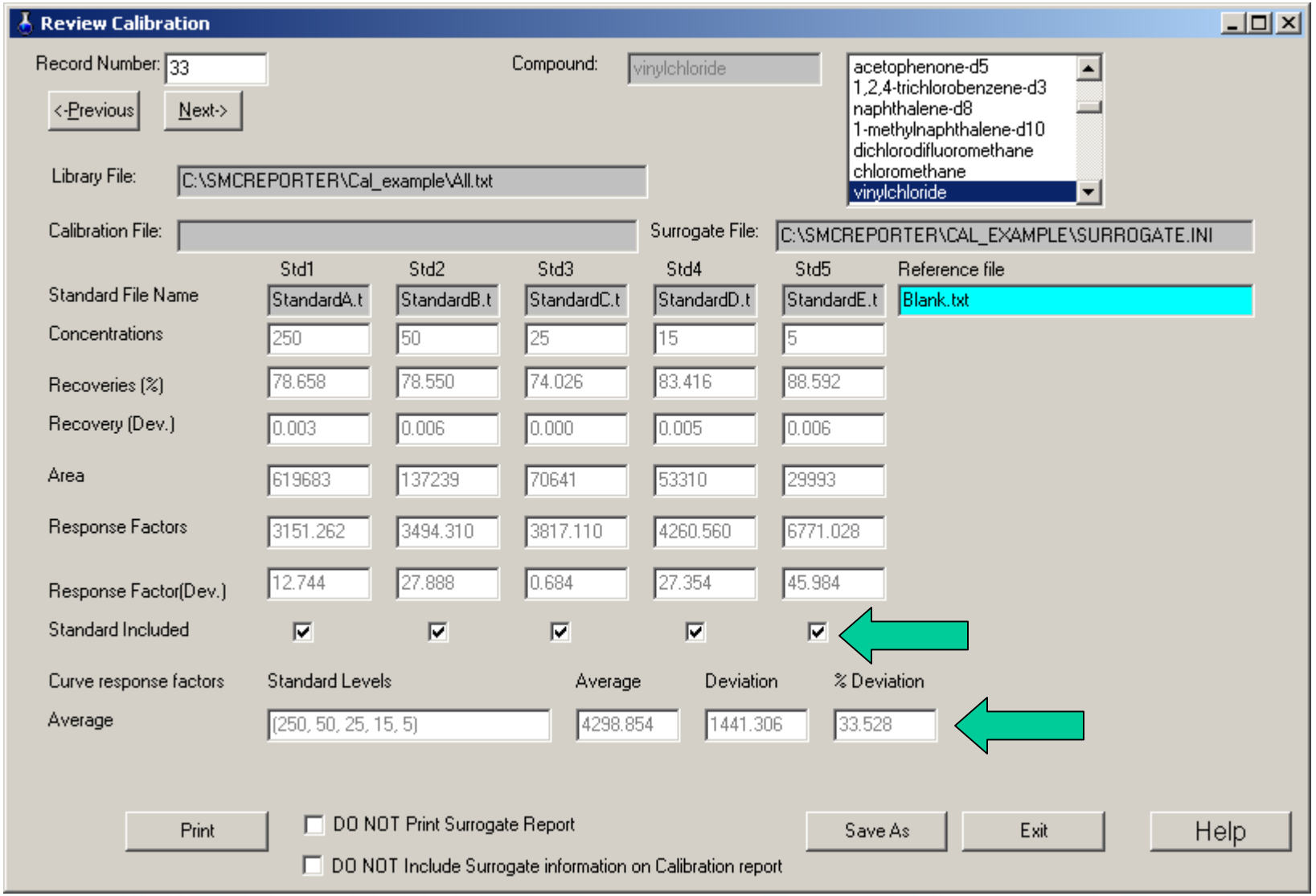
Review Calibration Form



Reviewing Calibration by Compound

- By selecting the “Next” tab (upper left corner) each compound is displayed.
- An experimental surrogate, aniline-$^{13}C_6$, was used in this calibration. This surrogate is not present in the available surrogate mix.
- Lets review the results for vinyl chloride. This can be done by entering “33” in the upper left corner record number box or clicking “next” to advance to 33.

Eliminating a Data Point



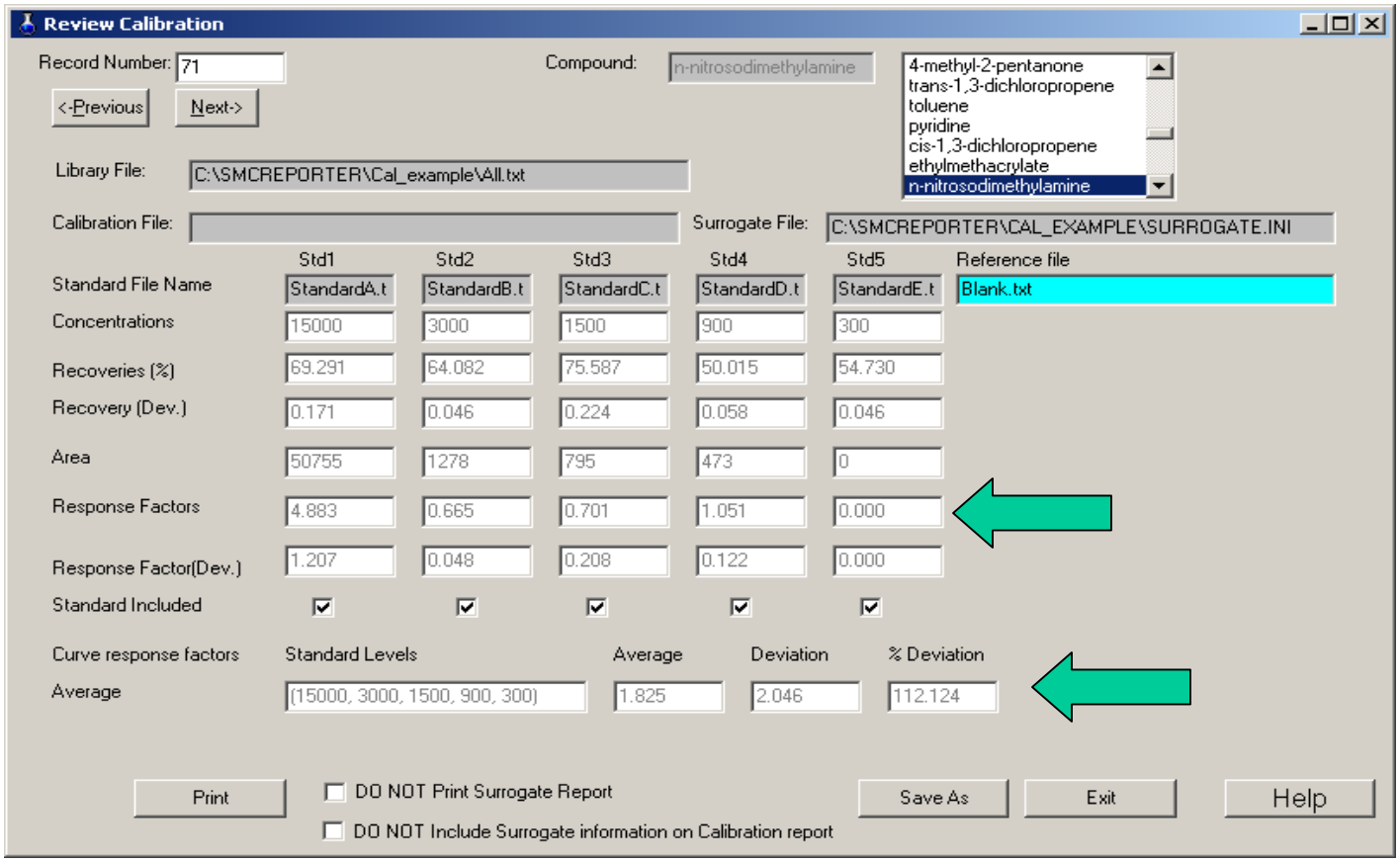
Editing the Calibration Curve

- The large deviation for vinyl chloride (see lower arrow on previous slide) indicates there is a problem with vinyl chloride.
- We can see that the lower standard (StandardE, 5ngs) has a much larger response factor than the other four standards. Evaluating the chromatogram for vinyl chloride for that compound we found that low level interference was causing the bias.
- For this exercise we will assume that the lower amount of vinyl chloride is below all required limits and the standard data point is not necessary. Remove the “Standard Included” check on Standard E by clicking on it (see upper arrow on previous slide).
- By removing the data point the linearity will be recalculated. Also the change will be recorded in the calibration file and the lower calibration limit will become 15 ngs/sample.

More Changes!

- The next two compounds that have large linearity deviations are common laboratory contaminants, Acetone (#38) and Methylene chloride (#43). The contribution due to background is observed in the elevated response factors for the lowest standards.
- For acetone remove data points for Standards D (30ng) and E (15 ng).
- For methylene chloride (#43) is also quite common. Remove data point for Standard E (5 ng/sample).
- Next advance to N-nitrosodimethylamine (#71). This compound chromatographs poorly on volatile organic compound capillary columns under most circumstances. (See next slide).

N-Nitrosodimethylamine



N-Nitrosodimethylamine (cont)

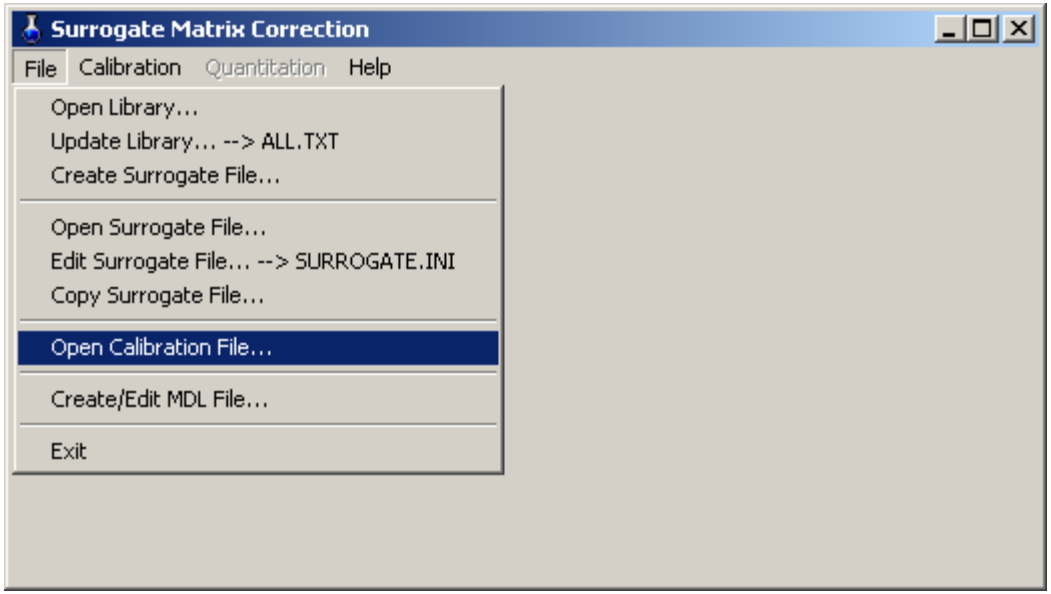
- Here, only the most concentrated standard (A) gives a response for the compound.
- Remove the “Standard Included” checks for standards B-E. This gives only a single data point. Other systems may give wider calibration range for this compound.
- Similarly, 2-picoline (#77), n-nitrosomethylethylamine (#82) and n-nitrosodipropylamine should be edited to give a single point.
- Aniline (#102) co-elutes with aniline-$^{13}C_6$ and the lower calibration levels are obscured by the surrogate. The two lower points should be removed. In the absence of the experimental co-eluting surrogate, calibration should be improved.

Save calibration curve

- After the review, save the calibration curve. Click on the “Save as” button on the lower right hand corner of the review and save the file as “test.cal”.
- The calibration file is a standalone file containing all information necessary to process sample data. If the SMCReporter program is closed test.cal can be reloaded for sample processing. “All.txt” and “surrogate.ini” do not have to be recalled.

Quantitate samples

- The newly created calibration file can now be used to process data.
- Load the calibration file by File->Open Calibration File



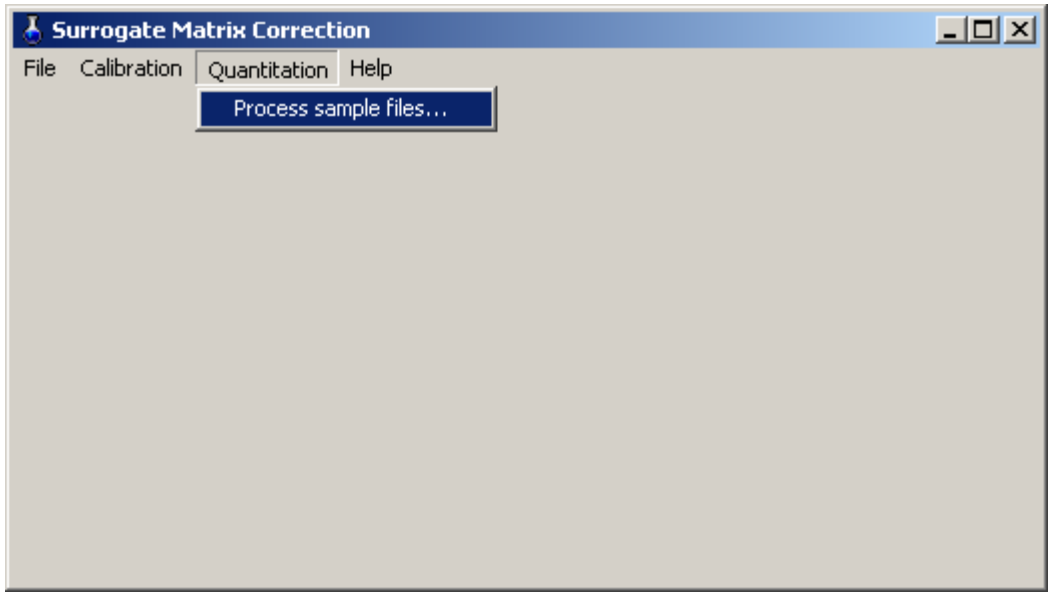
- Select the newly created test.cal

Calibration Loaded

- Notice that after loading the calibration file that library All.txt and surrogate.ini are disabled (under File).
- The main window (see slide 4) now has Calibration and Quantitation menu options enabled.
- In the Calibration menu options for review, printing, and check standard reporting are now enabled.

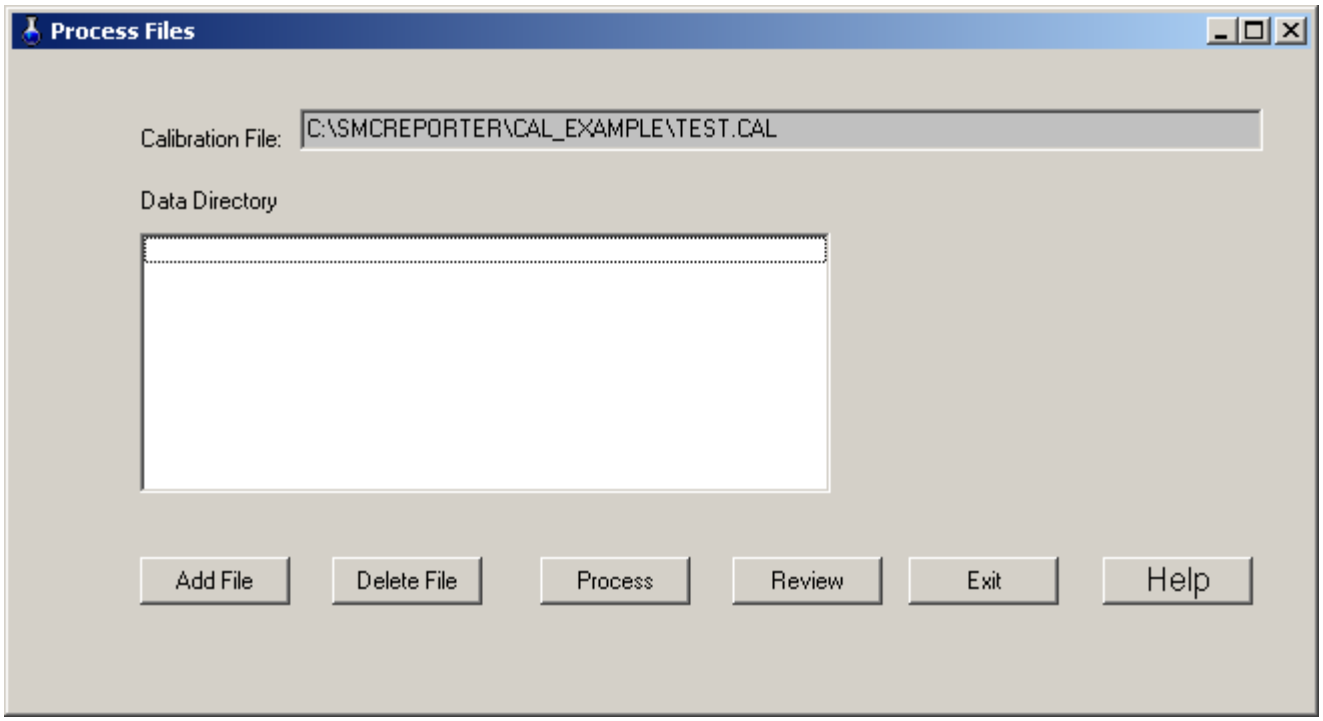
Quantitation

- With calibration file test.cal loaded the next step is to start the quantitation process.
- Select Quantitation->Process Sample Files



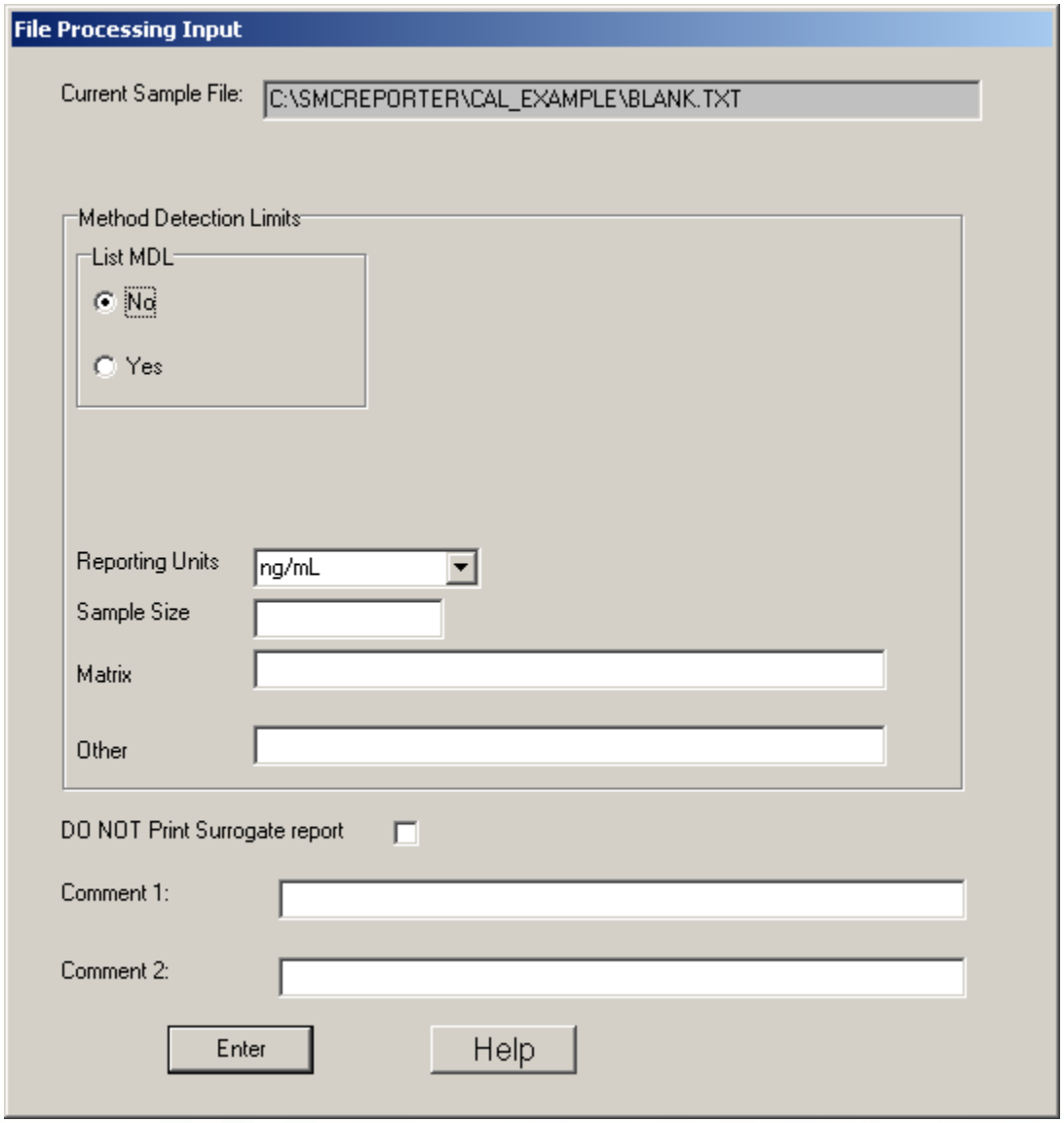
- The Process Files window for entering samples appears (next slide).

Enter File for Quantitation



- Select “Add File” and navigate to the blank used in calibration (Blank.txt)
- Upon selection of file a window for inputting sample information appears (next slide).

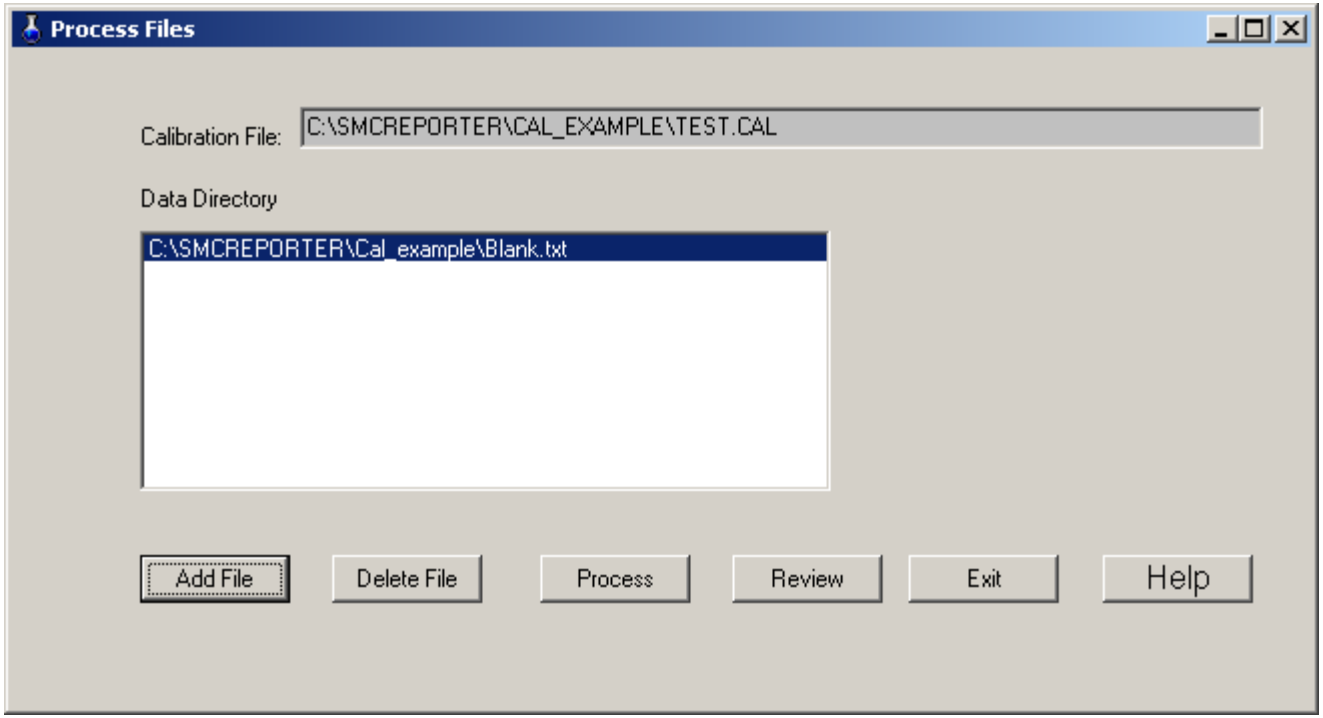
Input Window for File Processing



Sample Information

- Leave the “List MDL” “no” checked.
- Leave the reporting units as ng/mL.
- Enter 5 for the sample size (5 mL).
- Enter “Water” for matrix.
- The fields “other”, and “comments” 1 and 2 are for entering sample specific data.
- Check the “DO NOT print surrogate report” box. We will address that report later.
- Click “Enter”. The next window should look like the following slide.

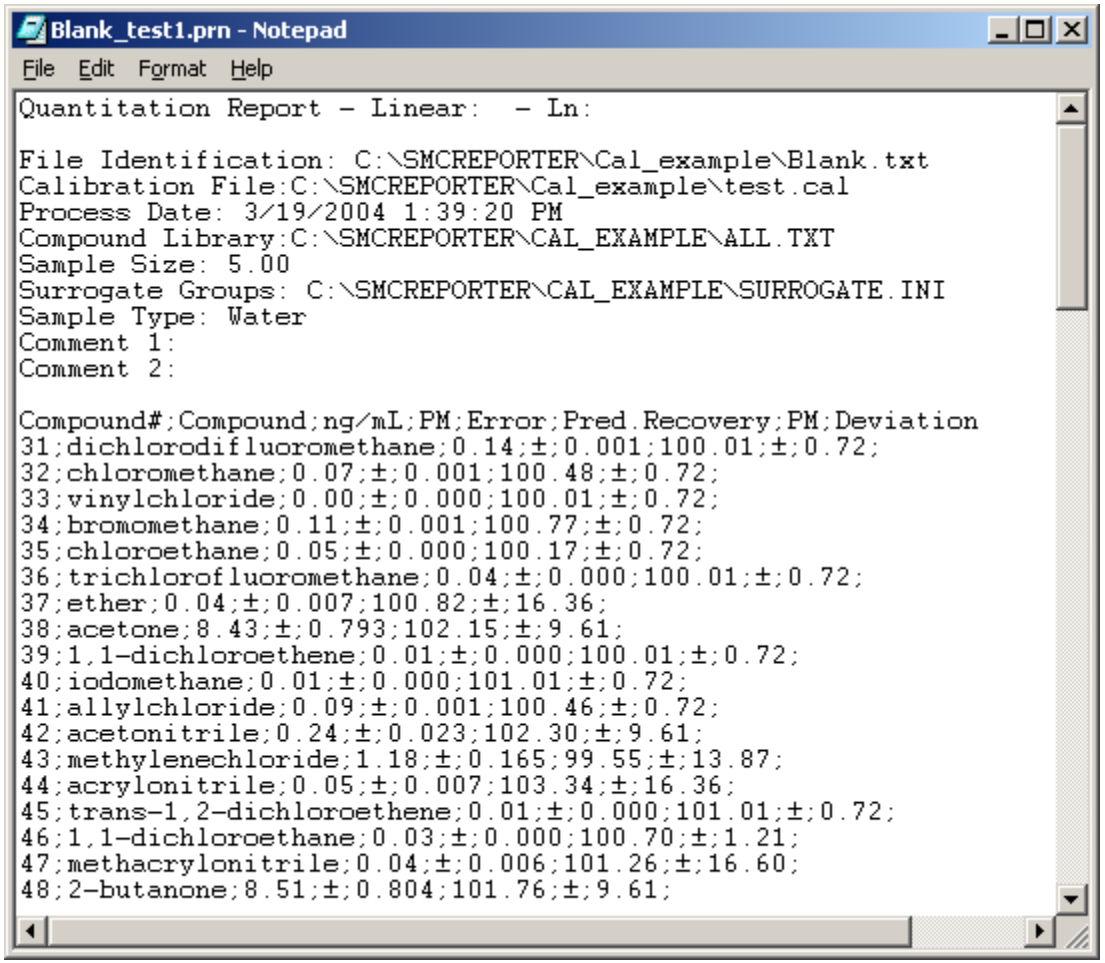
Sample Ready to Process



- For this exercise we will only process the blank. The standard runs can also be quantitated like samples if desired.
- Now click “Process” and a quantitation report (both paper and electronic) will be generated for the sample.

Electronic Data Report

- The file name of the report is Blank_test1.prn and resides in the same folder Blank.txt resides. The “test1” represents the calibration “test.cal” and the first quantitation of the file using test.cal.



Quantitation Report

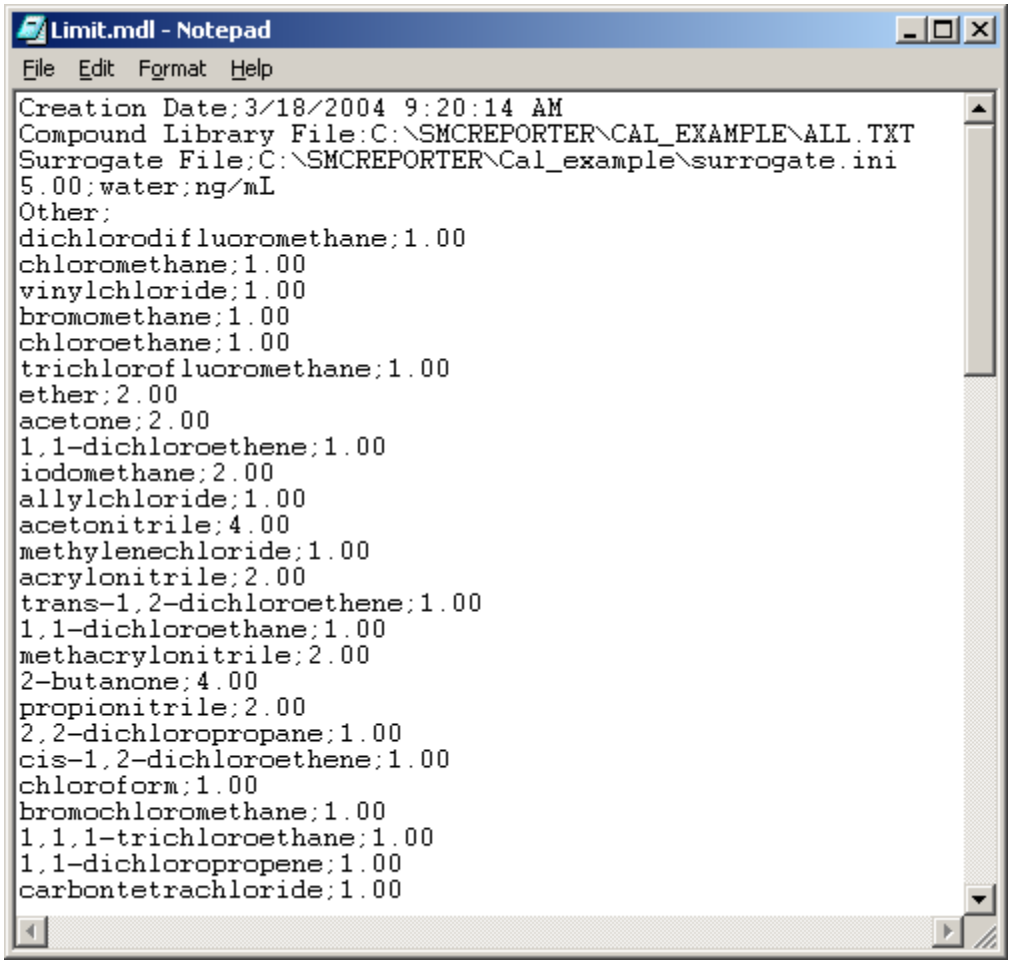
- Note the header contains a description of all components that were used to generate the report and the file path.
- Each compound is listed with its calculated concentration, method error, predicted recovery and deviation.
- The paper copy of the report contains the same information but with a presentation more compatible with a paper display.
- Normally results are not reported if they do not exceed some limit such as method detection limit (MDL). addressed in the following slide.

MDL data

- The MDL option on the File Processing Input window (slide #31) is the means to limit results to only those above a threshold (method detection limit, reporting limit, lower calibration point).
- A limit file was created for this presentation as the the concentration of the lower calibration point in 5 mL water (Limit.mdl).
- The adventurous can create their own limit file. This requires reloading the library, All.txt, and the surrogate file, surrogate.ini. Then Menu->File->Create/Edit MDL File. Type of limit file (matrix and sample size) are entered and compound limits be entered individually or through a paste command. See the SMCReporter operating manual for greater detail.
- The next slide presents the format of the file (Limit.mdl).

Limit.txt

- The limit file is a ";" delimited *.mdl file.
- The header contains information to identify what sample size, matrix, library and surrogate are related to the limit.
- Labeling limit files should include pertinent information such as "5mLwatermdl_inst1.mdl" or "5mLreportlimit.mdl" for ease of locating.

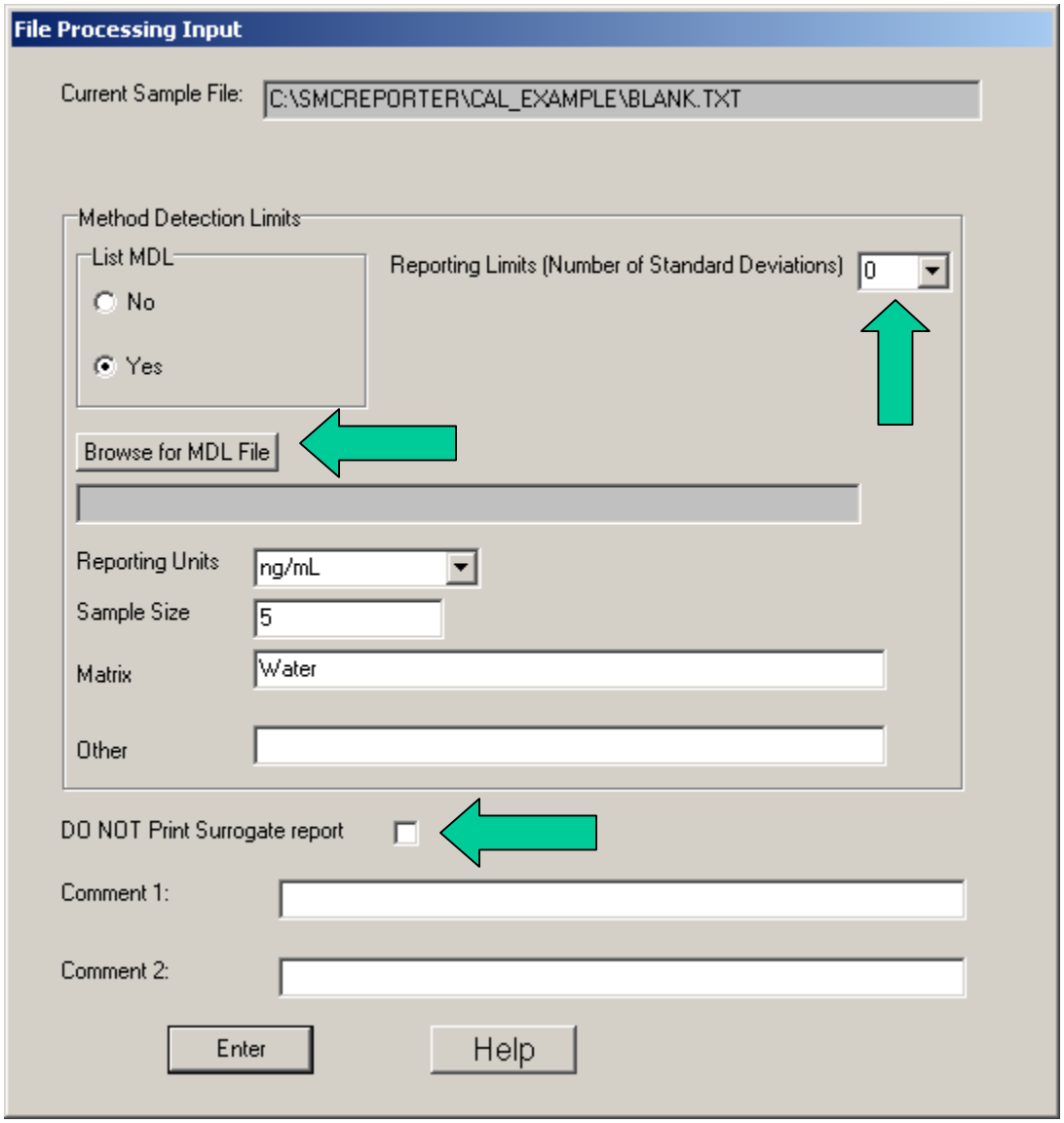


Process Files with a Reporting Limit

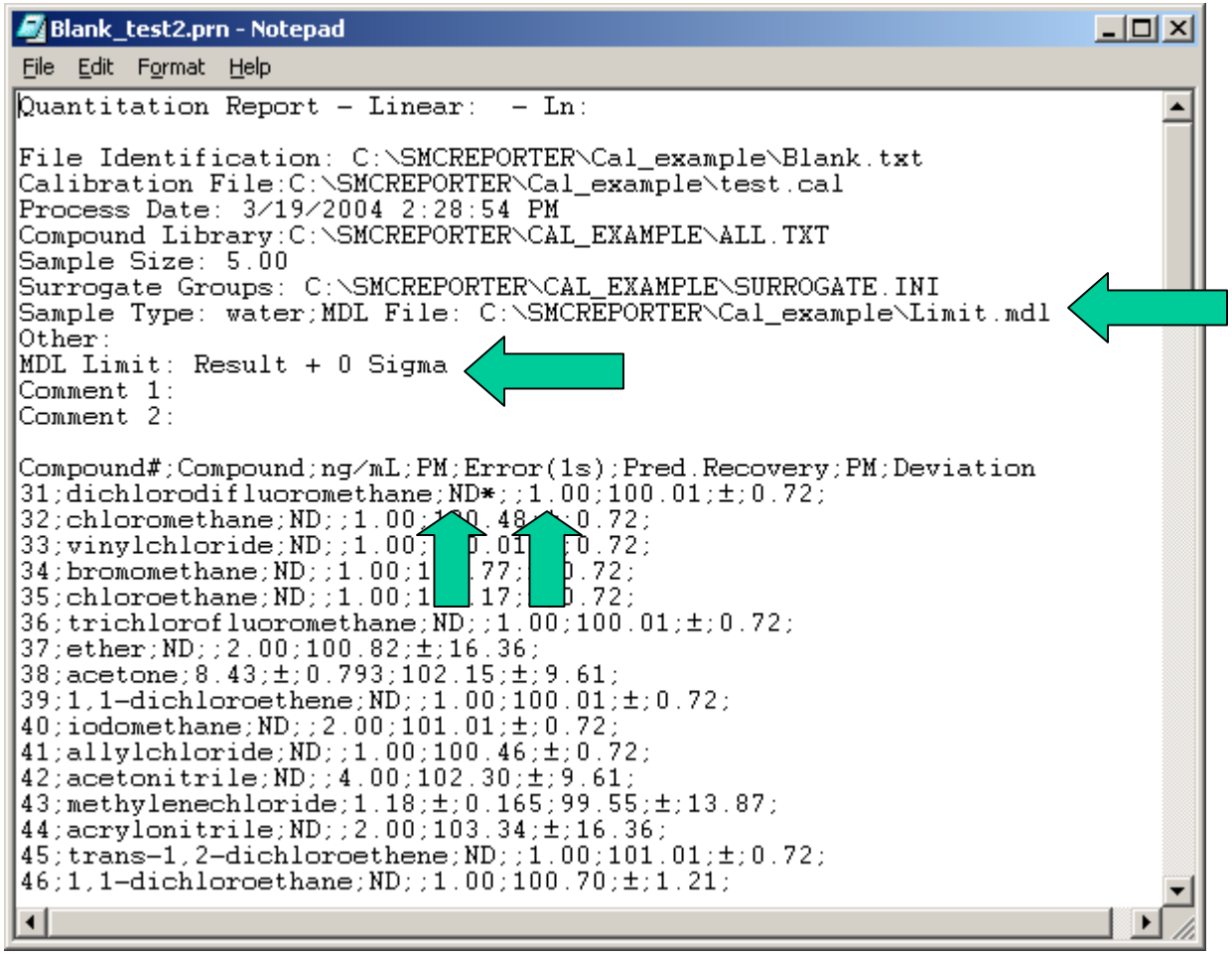
- To observe the use of limits return to the data processing routine (slide 29) and re-enter the Blank.txt. When the "File Processing Input" window is displayed check "yes" in the "List MDL" option.
- Note that the window retains the previous inputs as new defaults.

Input with Limit Option

- Use browse to locate the MDL file “Limit.mdl” and load.
- The “DO NOT Print Surrogate report” should not be checked. We will look at the surrogate files this time.
- For now the “Reporting Limits” box remains “0”.
- Click “Enter”.
- Then click “Process” on the returned Process Files window.



Electronic Data Report with Limits



Quantitation Report with Limits

- The electronic version name is Blank_test2.prn with the “2” indicating this is the second quantitation of the file using test.cal.
- The reprocessed quantitation report now displays the results with “ND” for not detected below the limit (Limit.mdl is in the header)
- The limit values are now presented in the “Error” column.
- The paper version of the report contains the same information as the new electronic version but in a different layout as before.

Reporting Limits Including Method Precision

- Note that in the header there is a MDL limit: with “Result + 0 Sigma”. This is the “0” value in “Reporting Limits (number of standard deviations)” from the File Process Input window. This value is included in reporting values and including the method error. If “3” is entered in the input window, a compound concentration will be reported if the upper limit of 3x the method error is $\geq$ the limit.
- Reprocess the Blank.txt file with the reporting limits deviation at “3”. **Note: the method error reported with concentrations is still only 1 deviation**.
- A concentration (in ng/mL) for aniline will be displayed as 45.6 $\pm$ 6.47 and not “ND”. This is because the aniline concentration + 3Xdeviation exceed the limit (45.6 + 3X6.47 = 65.01 > the 60 limit value).

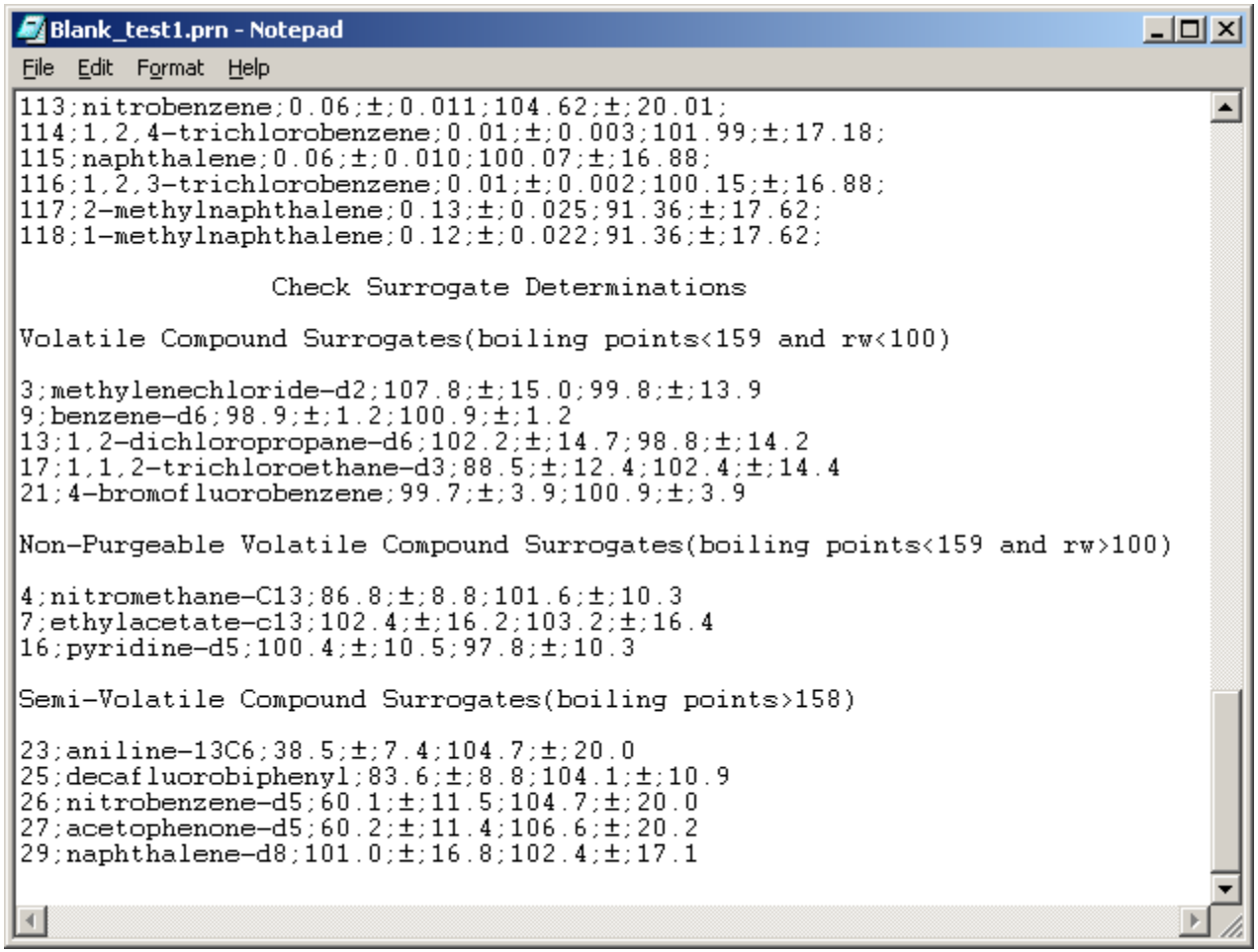
QC report

- The QC report contains the equations used to generate the results (paper copy presents a graphic presentation of effects observed).
- The QC report contains the method 8261 calculations on the monitoring “check” surrogates as individual compounds and as volatile, non-purgeable volatile, and semivolatile groupings.
- With the exception of graphics the electronic version of the QC report contains the same data and this discussion will reference the electronic version.

Electronic QC Report

- The check surrogates are presented following the listing of the compound results in the data file. See next slide. The check surrogate results are also presented in a separate QC file.
- The electronic QC report is included in a *.SQL file. To read the file use notepad and open Blank_test2.SQC.
- The Blank_test2.SQL file is displayed in the slide #46.

Electronic Version of Check Surrogates



QC Report-Electronic Format

Blank_test2.SQC - Notepad

File Edit Format Help

```
Surrogate Report
Process Date;3/19/2004 2:28:54 PM
Compound Library File;C:\SMCREPORTER\CAL_EXAMPLE\ALL.TXT
Surrogate Groupings File;C:\SMCREPORTER\CAL_EXAMPLE\SURROGATE.INI
C:\SMCREPORTER\CAL_EXAMPLE\BLANK.TXT
Calibration File;C:\SMCREPORTER\Cal_example\test.cal

First Pass relative Volatility (Ln) vs. Recovery
(Used to Estimate Rel vol effects on BP Surrogates)

Compound;;;Measured;Recovery
hexafluorobenzene;81.5;0.86;99.98;99.98
Fluorobenzene;85.0;3.50;100.38;100.38

Slope = 0.002884;Intercept = 1.000215;Error = 0.000;RSQ = 0.000000

Fluorobenzene;85.0;3.50;100.38;100.38
1,2-dichloroethane-d4;84.0;20.00;95.27;95.27

Slope = -0.029346;Intercept = 1.040591;Error = 0.000;RSQ = 0.000000


Recovery vs. Boiling Point - (First Pass Rel Vol Corrections)-- Boiling point range( 85.00 - 231.00)
Compound;;;Measured;Recovery
pentafluorobenzene;85.0;1.51;100.49;100.35
toluene-d8;111.0;4.28;96.49;96.70
bromobenzene-d5;155.0;7.93;100.91;102.99

Slope = 0.000488;Intercept = 0.943014;Error = 0.037;RSQ = 0.000011

bromobenzene-d5;155.0;7.93;100.91;102.99
```

Summary

- All data is generated in paper and electronic format.
- QC is generated for each sample.
- There are options for limits to report data.
- Data generated can be read into other programs for customizing reports.

Note:The SMCReporter will be updated periodically to add new features.